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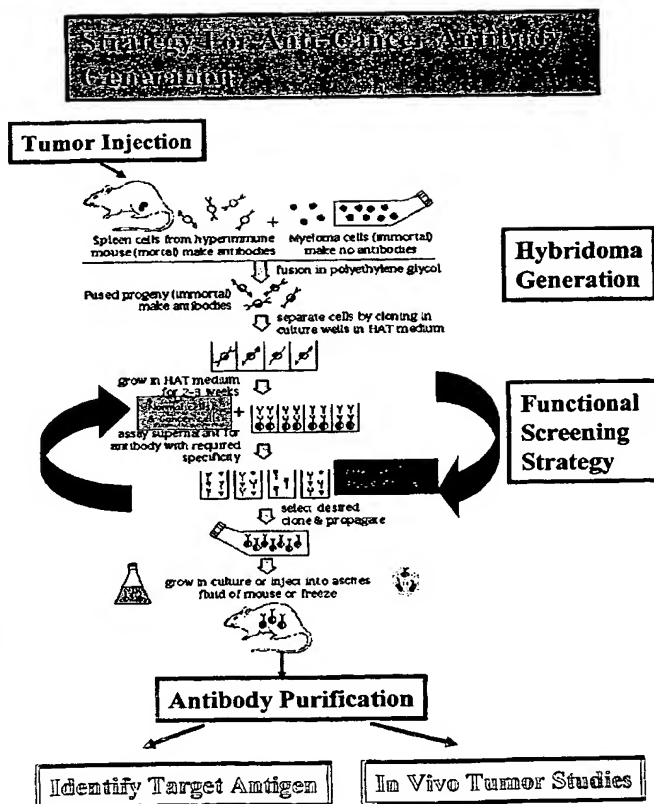
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(54) Title: ANTIGENIC PROFILING OF NEOPLASTIC CELLS, ONCOGENIC THERAPY UTILIZING FUNCTIONAL ANTIBODIES THERETO AND CYTOTOXIC IMMUNE COMPLEXES FORMED THEREBY



(57) Abstract: The present invention relates to a method for producing patient specific anti-cancer antibodies using a novel paradigm of screening and characterizing the target antigen thereof. The invention further relates to the process by which the antibodies are made and to their methods of use. The antibodies can be used in aid of staging and diagnosis of a cancer, and can be used to treat primary tumors and their metastases. The anti-cancer antibodies can be combined with a variety of anti-neoplastic agents, e.g. Cisplatin, to derive a synergistic effect with regard to reduction in tumour growth kinetics and metastasis.

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**ANTIGENIC PROFILING OF NEOPLASTIC CELLS, ONCOGENIC THERAPY**  
**UTILIZING FUNCTIONAL ANTIBODIES THERETO AND**  
**CYTOTOXIC IMMUNE COMPLEXES FORMED THEREBY**

**Reference to Related Applications:**

This application relates to S.N. 09/727,361, filed November 29, 2000, and U.S. Patent 6,180,357, the contents of which are herein incorporated by reference.

**Field of the Invention:**

This application is directed toward the use of functional antibodies having the ability to bind to an epitope of Cytokeratin 18 (CK18) either alone or as an obligate heterodimer in combination with Cytokeratin 8 (CK8); to detection, localization and treatment of neoplastic cells whose cell membrane possesses an antigenic profile embodying the required binding site necessary for formation of a cytotoxic immune complex upon binding with said functional monoclonal antibodies, and to oncogenic therapy of said cells via utilization of said functional antibodies either alone or in combination with traditional neoplastic agents.

**Background of the Invention:**

Each individual who presents with cancer is unique and has a cancer that is as different from other cancers as that person's identity. Despite this, current therapy treats all patients with the same type of cancer, at the same stage, in the same way. At least 30% of these patients will fail the first line therapy, thus leading to further rounds of treatment and the increased probability of treatment failure, metastases, and ultimately, death. A superior approach to treatment would be the customization of therapy for the particular individual. The only current therapy which lends itself to customization is surgery. Chemotherapy and radiation treatment can not be tailored to the patient, and surgery

1 by itself, in most cases is inadequate for producing  
2 cures.

3       The development of anti-cancer monoclonal antibodies  
4 (mAbs) has raised the possibility of tailoring them for  
5 personalized therapy based on the antigenic profile of the  
6 patient's tumor. By making use of a method for producing  
7 functional anti-cancer mAbs through the use of patient  
8 biopsy samples and a novel paradigm of screening (in  
9 accordance with the teachings of U.S. 6,180,357), a  
10 library of anti-cancer mAbs may be generated that can be  
11 used alone, in combination with other mAbs based on the  
12 expression of antigens on the patient's tumor, or  
13 furthermore in combination with traditional neoplastic  
14 agents in the hope of deriving an additive or possibly  
15 even a synergistic effect.

16       Having recognized that a significant difference  
17 between cancerous and normal cells is that cancerous cells  
18 contain antigens that are specific to transformed cells,  
19 the scientific community has long held that monoclonal  
20 antibodies can be designed to specifically target  
21 transformed cells by binding specifically to these cancer  
22 antigens; thus giving rise to the belief that monoclonal  
23 antibodies can serve as "Magic Bullets" to eliminate  
24 cancer cells.

25 **Prior Art:**

26       U.S. Patent No. 5,750,102 discloses a process wherein  
27 cells from a patient's tumor are transfected with MHC  
28 genes which may be cloned from cells or tissue from the  
29 patient. These transfected cells are then used to  
30 vaccinate the patient.

31       U.S. Patent No. 4,861,581 discloses a process  
32 comprising the steps of obtaining monoclonal antibodies  
33 that are specific to an internal cellular component of  
34 neoplastic and normal cells of the mammal but not to  
35 external components, labeling the monoclonal antibody,

1 contacting the labeled antibody with tissue of a mammal  
2 that has received therapy to kill neoplastic cells, and  
3 determining the effectiveness of therapy by measuring the  
4 binding of the labeled antibody to the internal cellular  
5 component of the degenerating neoplastic cells. In  
6 preparing antibodies directed to human intracellular  
7 antigens, the patentee recognizes that malignant cells  
8 represent a convenient source of such antigens.

9 U.S. Patent No. 5,171,665 provides a novel antibody  
10 and method for its production. Specifically, the patent  
11 teaches formation of a monoclonal antibody which has the  
12 property of binding strongly to a protein antigen  
13 associated with human tumors, e.g. those of the colon and  
14 lung, while binding to normal cells to a much lesser  
15 degree.

16 U.S. Patent No. 5,484,596 provides a method of cancer  
17 therapy comprising surgically removing tumor tissue from a  
18 human cancer patient, treating the tumor tissue to obtain  
19 tumor cells, irradiating the tumor cells to be viable but  
20 non-tumorigenic, and using these cells to prepare a  
21 vaccine for the patient capable of inhibiting recurrence  
22 of the primary tumor while simultaneously inhibiting  
23 metastases. The patent teaches the development of  
24 monoclonal antibodies which are reactive with surface  
25 antigens of tumor cells. As set forth at col. 4, lines 45  
26 et seq., the patentees utilize autochthonous tumor cells  
27 in the development of monoclonal antibodies expressing  
28 active specific immunotherapy in human neoplasia.

29 U.S. Patent No. 5,725,856 is directed toward  
30 treatment of carcinomas which overexpress HER2 receptor  
31 comprising administration of an antibody which binds to  
32 the extracellular domain of the HER2 receptor, thereby  
33 reducing or eliminating a patient's tumor burden. The  
34 patent teaches conjugation of the antibody to a cytotoxic  
35 moiety.

1 U.S. Patent No. 5,783,186 is drawn to Anti-Her2  
2 antibodies which induce apoptosis in Her2 expressing  
3 cells, hybridoma cell lines producing the antibodies,  
4 methods of treating cancer using the antibodies and  
5 pharmaceutical compositions including said antibodies.

6 U.S. Patent No. 5,849,876 describes new hybridoma  
7 cell lines for the production of monoclonal antibodies to  
8 mucin antigens purified from tumor and non-tumor tissue  
9 sources.

10 U.S. Patent No. 5,869,045 relates to antibodies,  
11 antibody fragments, antibody conjugates and single chain  
12 immunotoxins reactive with human carcinoma cells. The  
13 mechanism by which these antibodies function is two-fold,  
14 in that the molecules are reactive with cell membrane  
15 antigens present on the surface of human carcinomas, and  
16 further in that the antibodies have the ability to  
17 internalize within the carcinoma cells, subsequent to  
18 binding, making them especially useful for forming  
19 antibody-drug and antibody-toxin conjugates. In their  
20 unmodified form the antibodies also manifest cytotoxic  
21 properties at specific concentrations.

22 U.S. Patent No. 6,207,153 is directed towards antigen  
23 binding fragments recognized by H11, described as the C-  
24 antigen, nucleotides encoding the fragments, and their use  
25 for prophylaxis and detection of cancers.

26 Eto et al, Mapping and Regulation of the Tumor-  
27 associated Epitope Recognized by Monoclonal Antibody RS-11  
28 (Journal of Biological Chemistry, Vol. 275, No. 35,  
29 9/2000, Pp. 27075-27083) discloses an antibody recognized  
30 by a tumor-associated antigen. The RS-11 antibody appears  
31 to recognize an epitope of Keratin 18 and/or Keratin 8  
32 expressed in neoplastic cells, but not present in normal  
33 cells. In contrast, the antibody of the instant  
34 invention, ARH460-23, appears to bind with an epitope of  
35 CK18 within the cytoplasm or perinuclear region of normal

1 cells, while only recognizing a binding site residing upon  
2 the cellular membrane of neoplastic cells.

3 Oshima et al, "Oncogenic Regulation and Function of  
4 Keratins 8 and 18" (Cancer and Metastasis Reviews 15:445-  
5 471, 1996, disclose the widespread expression of CK8 and  
6 CK18 in carcinoma cells, and indicate that they are a  
7 useful tool in the understanding of cancer and metastasis.

8

9 **Summary of the Invention:**

10 A variety of therapeutic anti-cancer monoclonal  
11 antibodies (mAbs) are commonly generated involving a  
12 strategy whereby mAbs are formed against a single target  
13 and screened for their ability to bind a well-defined  
14 tumor-associated antigen. Furthermore it is known to  
15 generate functional therapeutic anti-cancer mAbs by using  
16 tumor cells as immunogens to provide an extensive array of  
17 target antigens and to screen for anti-cancer activity  
18 instead of binding. The present invention has utilized a  
19 method in accordance with U. S. Patent No. 6,180,357,  
20 whereby anti-cancer mAbs are produced through the use of  
21 patient biopsy samples and a novel model of screening that  
22 selects for antibody-producing clones that discriminately  
23 kill tumor cells and not normal cells. This strategy was  
24 used to generate a plurality of mAbs, from which a  
25 particular antibody designated ARH460-23, a murine IgM,  
26 kappa mAb that was generated in response to a lung tumor  
27 biopsy, was selected for further study. The binding  
28 activity of ARH460-23 is characterized in accordance with  
29 the instant invention and the in vivo efficacy of ARH460-  
30 23 as an anti-tumor agent, having a particular activity  
31 against the lung cancer line, NCI H460 is demonstrated.  
32 The ARH460-23 antigen is identified as the well-defined  
33 tumor-associated antigen, cytokeratin 18. The methodology  
34 employed herein creates an opportunity for generating

6

1 functional mAbs towards tumor-associated antigens using  
2 tumor tissue, instead of a defined target.

3 The anti-cancer antibodies of the instant invention  
4 may be used alone, in combination with other mAbs based on  
5 the expression of antigens on the patient's tumor, or  
6 alternatively in combination with other neoplastic agents,  
7 for example radioisotopes, vinca alkaloids, adriamycin,  
8 bleomycin sulfate, Carboplatin, Cisplatin,  
9 cyclophosphamide, Cytarabine, Dacarbazine, Dactinomycin,  
10 Duanorubicin hydrochloride, Doxorubicin hydrochloride,  
11 Etoposide, fluorouracil, lomustine, Mechlorethamine  
12 hydrochloride, melphalan, mercaptopurine, methotrexate,  
13 mitomycin, mitotane, pentostatin, pipobroman procarbaze  
14 hydrochloride, streptozotocin, taxol, thioguanine, Uracil  
15 mustard, and the like.

16 Antibodies produced in the context of the present  
17 invention, were generated in mice immunized with fixed  
18 cells from one of several types of tumor biopsies.  
19 Functional mAbs were identified using a selective  
20 screening process and evaluated for in vitro cytotoxicity  
21 towards tumor cells, in vivo anti-tumor activity and  
22 tissue specificity. Investigational techniques were  
23 utilized to determine the formation of immune complexes  
24 and the antigenic target of the antibody was thereby  
25 determined.

26 Within the context of this application, anti-cancer  
27 antibodies having either cell-killing (cytotoxic) or cell-  
28 growth inhibiting (cytostatic) properties will hereafter  
29 be referred to as cytotoxic. These antibodies can be used  
30 in aid of staging and diagnosis of a cancer, and can be  
31 used to treat tumor metastases. The antibodies may be  
32 conjugated to other hematogenous cells, e.g. lymphocytes,  
33 macrophages, monocytes, natural killer cells, etc.

34

35 Accordingly, it is an objective of the invention to



1 teach one or more novel anti-cancer antibodies which are  
2 cytotoxic with respect to cancer cells while  
3 simultaneously being relatively non-toxic to non-  
4 cancerous cells.

5 It is an additional objective of the invention to  
6 identify and characterize the antigenic target for the  
7 anti-cancer antibody.

8 It is yet an additional objective of the instant to  
9 determine the point of formation, at a sub-cellular level,  
10 of the immune complex, wherein said point of formation  
11 evidences an oncogenic variation in the cell.

12 A still further objective of the instant invention is  
13 to produce anti-cancer antibodies which are useful for  
14 diagnosis, prognosis, localization, and signaling of  
15 oncogenic change at a cellular level.

16 It is yet an additional objective of the instant  
17 invention to treat patients having primary cancers which  
18 express CK18 and metastatic tumors thereof.

#### 19 **Brief Description of the Figures**

20 Figure 1 is an outline of a strategy for Anti-Cancer  
21 Antibody generation, purification, and target antigen  
22 identification;

23 Figure 2 describes metastasis reduction in an orthotopic  
24 implantation model;

25 Figure 3 is a 2-D gel analysis of NCI H460 membrane  
26 proteins probed with ARH460-23;

27 Figure 4 is a 2-D gel analysis of membrane proteins from  
28 Jurkat cells probed with ARH460-23;

29 Figure 5 is a graphical representation of flow cytometric  
30 analysis showing differential reactivity of ARH460-23 for  
31 Jurkat and NCI H460 cells;

32 Figure 6 portrays the effect of preventative therapy on  
33 tumour growth kinetics utilizing a combination of antibody  
34 and anti-neoplastic agent;

8

1 Figure 7 is a tabular analysis of an immunohistochemistry  
2 study carried out on formalin-fixed, paraffin-embedded  
3 human tissues to profile expression of the ARH460-23  
4 antigen on normal and tumor tissues;  
5 Figure 8 represents the result of immunohistochemical  
6 staining of ARH460-23 with NCI H460 and Jurkat cell  
7 pellets.

8 **Detailed Description of the Invention:**

9 It is to be understood that while a certain form of  
10 the invention is illustrated, it is not to be limited to  
11 the specific form or arrangement herein described and  
12 shown. It will be apparent to those skilled in the art  
13 that various changes may be made without departing from  
14 the scope of the invention and the invention is not to be  
15 considered limited to what is shown and described in the  
16 specification.

17 Other objects and advantages of this invention will  
18 become apparent from the following description wherein are  
19 set forth, by way of illustration and example, certain  
20 embodiments of this invention.

21 Traditionally, monoclonal antibodies have been made  
22 according to fundamental principles laid down by Kohler  
23 and Milstein. Mice are immunized with antigens, with or  
24 without, adjuvants. The splenocytes are harvested from  
25 the spleen for fusion with immortalized hybridoma  
26 partners. These are seeded into microtitre plates where  
27 they can secrete antibodies into the supernatant that is  
28 used for cell culture. To select from the hybridomas that  
29 have been plated for the ones that produce antibodies of  
30 interest the hybridoma supernatants are usually tested for  
31 antibody binding to antigens in an ELISA (enzyme linked  
32 immunosorbent assay) assay. The idea is that the wells  
33 that contain the hybridoma of interest will contain  
34 antibodies that will bind most avidly to the test antigen,  
35 usually the immunizing antigen. These wells are then

1 subcloned in limiting dilution fashion to produce  
2 monoclonal hybridomas. The selection for the clones of  
3 interest is repeated using an ELISA assay to test for  
4 antibody binding. Therefore, the principle that has been  
5 propagated is that in the production of monoclonal  
6 antibodies the hybridomas that produce the most avidly  
7 binding antibodies are the ones that are selected from  
8 among all the hybridomas that were initially produced.  
9 That is to say, the preferred antibody is the one with  
10 highest affinity for the antigen of interest.

11 There have been many modifications of this procedure  
12 such as using whole cells for immunization. In this  
13 method, instead of using purified antigens, entire cells  
14 are used for immunization. Another modification is the  
15 use of cellular ELISA for screening. In this method  
16 instead of using purified antigens as the target in the  
17 ELISA, fixed cells are used. In addition to ELISA tests,  
18 complement mediated cytotoxicity assays have also been  
19 used in the screening process. However, antibody-binding  
20 assays were used in conjunction with cytotoxicity tests.  
21 Thus, despite many modifications, the process of producing  
22 monoclonal antibodies relies on antibody binding to the  
23 test antigen as an endpoint.

24 Most antibodies directed against cancer cells have  
25 been produced using the traditional methods outlined  
26 above. These antibodies have been used both  
27 therapeutically and diagnostically. In general, for both  
28 these applications, the antibody has been used as the  
29 targeting agent that delivers a payload to the site of the  
30 cancer. These antibody conjugates can either be  
31 radioactive, toxic, or serve as an intermediary for  
32 further delivery of a drug to the body, such as an enzyme  
33 or biotin. Furthermore, it was widely held, until  
34 recently, that naked antibodies had little effect *in vivo*.  
35 Both HERCEPTIN and RITUXIMAB are humanized murine

11

1 killing need not be predicated upon screening of the  
2 hybridomas for the best binding antibodies. Rather,  
3 although not advocated by those who produce monoclonal  
4 antibodies, the screening of the hybridoma supernatants  
5 for cell killing or alternatively for cessation of growth  
6 of the cancerous cells may be selected as a desirable  
7 endpoint for the production of cytotoxic or cytostatic  
8 antibodies. It is well understood that the *in-vivo*  
9 antibodies mediate their function through the Fc portions  
10 and that the utility of the therapeutic antibody is  
11 determined by the functionality of the constant region or  
12 attached moieties. In this case the FAb portion of the  
13 antibody, the antigen-combining portion, will confer to  
14 the antibody its specificity and the Fc portion its  
15 functionality. The antigen combining site of the antibody  
16 can be considered to be the product of a natural  
17 combinatorial library. The result of the rearrangement of  
18 the variable region of the antibody can be considered a  
19 molecular combinatorial library where the output is a  
20 peptide. Therefore, the sampling of this combinatorial  
21 library can be based on any parameter. Like sampling a  
22 natural compound library for antibiotics, it is possible  
23 to sample an antibody library for cytotoxic or cytostatic  
24 compounds.

25       The various endpoints in a screen must be  
26 differentiated from each other. For example, the  
27 difference between antibody binding to the cell is  
28 distinct from cell killing. Cell killing (cytotoxicity) is  
29 distinct from the mechanisms of cell death such as oncosis  
30 or apoptosis. There can be many processes by which cell  
31 death is achieved and some of these can lead either to  
32 oncosis or apoptosis. There is speculation that there are  
33 other cell death mechanisms other than oncosis or  
34 apoptosis but regardless of how the cell arrives at death  
35 there are some commonalities of cell death. One of these

1 monoclonal antibodies that have recently been approved for  
2 human use by the FDA. However, both these antibodies were  
3 initially made by assaying for antibody binding and their  
4 direct cytotoxicity was not the primary goal during the  
5 production of hybridomas. Any tendency for these  
6 antibodies to produce tumor cell killing is thus through  
7 chance, not by design.

8       Although the production of monoclonal antibodies have  
9 been carried out using whole cell immunization for various  
10 applications the screening of these hybridomas have relied  
11 on either putative or identified target antigens or on the  
12 selectivity of these hybridomas for specific tissues. It  
13 is axiomatic that the best antibodies are the ones with  
14 the highest binding constants. This concept originated  
15 from the basic biochemical principle that enzymes with the  
16 highest binding constants were the ones that were the most  
17 effective for catalyzing a reaction. This concept is  
18 applicable to receptor ligand binding where the drug  
19 molecule binding to the receptor with the greatest  
20 affinity usually has the highest probability for  
21 initiating or inhibiting a signal. However, this may not  
22 always be the case since it is possible that in certain  
23 situations there may be cases where the initiation or  
24 inhibition of a signal may be mediated through non-  
25 receptor binding. The information conveyed by a  
26 conformational change induced by ligand binding can have  
27 many consequences such as a signal transduction,  
28 endocytosis, among the others. The ability to produce a  
29 conformational change in a receptor molecule may not  
30 necessarily be due to the filling of a ligand receptor  
31 pocket but may occur through the binding of another extra  
32 cellular domain or due to receptor clustering induced by a  
33 multivalent ligand.

34       As disclosed in U.S. Patent 6,180,357, and outlined  
35 in Figure 1, the production of antibodies to produce cell

12

1 is the absence of metabolism and another is the  
2 denaturation of enzymes. In either case vital stains will  
3 fail to stain these cells. These endpoints of cell death  
4 have been long understood and predate the current  
5 understanding of the mechanisms of cell death.  
6 Furthermore, there is the distinction between cytotoxic  
7 effects where cells are killed and cytostatic effects  
8 where the proliferation of cells are inhibited.

9 In a preferred embodiment of the present invention,  
10 the assay is conducted by focusing on cytotoxic activity  
11 toward cancerous cells as an end point. In a preferred  
12 embodiment, a live /dead assay kit , for example the  
13 LIVE/DEAD<sup>7</sup> Viability/Cytotoxicity Assay Kit (L-3224) by  
14 Molecular Probes, is utilized. The Molecular Probes kit  
15 provides a two-color fluorescence cell viability assay  
16 that is based on the simultaneous determination of live  
17 and dead cells with two probes that measure two recognized  
18 parameters of cell viability C intracellular esterase  
19 activity and plasma membrane integrity. The assay  
20 principles are general and applicable to most eukaryotic  
21 cell types, including adherent cells and certain tissues,  
22 but not to bacteria or yeast. This fluorescence-based  
23 method of assessing cell viability is preferred in place  
24 of such assays as trypan blue exclusion, Cr release and  
25 similar methods for determining cell viability and  
26 cytotoxicity.

27 In carrying out the assay, live cells are  
28 distinguished by the presence of ubiquitous intracellular  
29 esterase activity, determined by the enzymatic conversion  
30 of the virtually nonfluorescent cell-permeant CALCEIN AM  
31 to the intensely fluorescent Calcein. The polyanionic dye  
32 Calcein is well retained within live cells, producing an  
33 intense uniform green fluorescence in live cells (ex/em  
34 ~495 nm/~515 nm). EthD-1 enters cells with damaged  
35 membranes and undergoes a 40-fold enhancement of

13

1 fluorescence upon binding to nucleic acids, thereby  
2 producing a bright red fluorescence in dead cells (ex/em  
3 ~495 nm/~635 nm). EthD-1 is excluded by the intact plasma  
4 membrane of live cells. The determination of cell  
5 viability depends on these physical and biochemical  
6 properties of cells. Cytotoxic events that do not affect  
7 these cell properties may not be accurately assessed using  
8 this method. Background fluorescence levels are inherently  
9 low with this assay technique because the dyes are  
10 virtually nonfluorescent before interacting with cells.

11 The antibodies are designed and can be used for  
12 therapeutic treatment of cancer in patients. Ideally the  
13 antibodies can be naked antibodies. They can also be  
14 conjugated to toxins. They can be used to target other  
15 molecules to the cancer. e.g. biotin conjugated enzymes.  
16 Radioactive compounds can also be used for conjugation.

17 The antibodies can be fragmented and rearranged  
18 molecularly. For example Fv fragments can be made; sFv-  
19 single chain Fv fragments; diabodies etc.

20 It is envisioned that these antibodies can be used  
21 for diagnosis, prognosis, localization and monitoring of  
22 cancer and oncogenic changes at a cellular or sub-cellular  
23 level. For example the patients can have blood samples  
24 drawn for shed tumor antigens which can be detected by  
25 these antibodies in different formats such as ELISA  
26 assays, rapid test panel formats etc. The antibodies can  
27 be used to stain tumor biopsies for the purposes of  
28 diagnosis. In addition a panel of therapeutic antibodies  
29 can be used to test patient samples to determine if there  
30 are any suitable antibodies for therapeutic use.

31 In isolation of ARH460-23 customized anti-cancer  
32 antibodies were produced to a patient's lung cancer cells,  
33 but cultured cells were used in the antibody development  
34 process to demonstrate the generality of the immunization  
35 process. The samples were prepared into single cell

14

1 suspensions and fixed for injection into mice. After the  
2 completion of three rounds of immunization with cells derived  
3 directly from a patient's lung cancer, the mice were  
4 immunized twice with a human lung large cell carcinoma cell  
5 line (NCI-H460). Hybridomas were produced from splenocytes  
6 and the supernatants were screened against a variety of  
7 cancer cell lines and normal cells in standard cytotoxicity  
8 assays. Those hybridomas that were reactive against cancer  
9 cell lines but were not reactive against normal non-  
10 transformed cells were selected for further propagation.  
11 Clones that were considered positive were ones that  
12 selectively killed the cancer cells but did not kill the non-  
13 transformed cells. The antibodies are characterized for a  
14 large number of biochemical parameters and then humanized for  
15 therapeutic use.

16 The lung tumor cells isolated and cell lines were  
17 cultured. Balb/c mice, A strain with H-2<sup>d</sup> haplotype from  
18 Charles River Canada, St. Constant, Quebec, Canada, female,  
19 7-8 week old, were immunized with the human lung cancer cells  
20 emulsified in an equal volume of either Freund's complete  
21 adjuvant (FCA) for the first immunization and then in  
22 Freund's incomplete adjuvant (FIA) for subsequent  
23 immunizations at 0, 21, 45 days with  $5 \times 10^5$  cells. The mice  
24 were immunized with fixed NCI H460 cells, which were prepared  
25 from NCI H460 cells grown in T-75 cell culture flask by  
26 scraping mono-layer cells into cell suspensions at 105, 150  
27 and 170 days. Immunized mice were sacrificed 3-4 days after  
28 the final immunization with NCI H460 cells, given intra-  
29 peritoneally, in phosphate buffered saline buffer (PBS), pH  
30 7.4. The spleens were harvested and the splenocytes were  
31 divided into two aliquots for fusion with Sp2/0 myeloma  
32 partners using the methods outlined in Example 1.

33 The screening was carried out 10 days after the fusion  
34 against NCI H460 cells and CCD-27SK. Antibodies were



1 characterized for binding to different cell lines with a  
2 cellular ELISA.

3 The wells that were considered positive were subcloned  
4 and the same screening process was repeated 9 days and 18  
5 days later. A number of monoclonal antibodies were produced  
6 in accordance with the method of the present invention,  
7 including that which produces the antibody designated ARH460-  
8 23. The ARH460-23 antibody is produced by a hybridoma cell  
9 line deposited on November 21, 2000 with the American Type  
10 Culture Collection at 10801 University Boulevard, Manassas,  
11 Va. having an ATCC Accession Number PTA-2700. Upon issuance  
12 of a patent, access to this cell line will not be withheld.

13 Clones were able to produce antibodies that had a  
14 greater than 15% killing rate for cancerous cells and at the  
15 same time some of the clones were able to produce less than  
16 eight percent killing of normal control fibroblasts.

17 The anti-cancer antibodies of the invention are useful  
18 for treating a patient with a cancerous disease when  
19 administered in admixture with a pharmaceutically acceptable  
20 adjuvant, for example normal saline, a lipid emulsion,  
21 albumen, phosphate buffered saline or the like and are  
22 administered in an amount effective to mediate treatment of  
23 said cancerous disease, for example with a range of about 1  
24 microgram per milliliter to about 1 gram per milliliter.

25 The method for treating a patient suffering from a  
26 cancerous disease may further include the use of conjugated  
27 anti-cancer antibodies and would thus include conjugating  
28 patient specific anti-cancer antibodies with a member  
29 selected from the group consisting of toxins, enzymes,  
30 radioactive compounds, and hematogenous cells; and  
31 administering these conjugated antibodies to the patient;  
32 wherein said anti-cancer antibodies are administered in  
33 admixture with a pharmaceutically acceptable adjuvant, for  
34 example normal saline, a lipid emulsion, albumen, phosphate  
35 buffered saline or the like and are administered in an amount

16

1 effective to mediate treatment of said cancerous disease, for  
2 example with a range of about 1 microgram per mil to about 1  
3 gram per mil. In a particular embodiment, the anti-cancer  
4 antibodies useful in either of the above outlined methods may  
5 be a humanized antibody.

6 An experiment to determine the effects of antibody  
7 administration in conjunction with anti-neoplastic agents was  
8 carried out and is summarized as follows:

## 9 MATERIALS AND METHODS

10

### 11 In Vivo Study Protocols

12

13

#### 14 Heterotopic Tumour Cell Engraftment

15 For all animals,  $1 \times 10^6$  cells (100  $\mu$ L of PBS containing  
16  $1 \times 10^7$  cell/mL) of the lung cancer cell line NCI-H460 were  
17 implanted subcutaneously into the scruff of the neck of seven  
18 week old female SCID mice.

19

#### 20 Treatment

21 The study consisted of 4 groups of 10 mice. Treatment  
22 was initiated 3 days after tumor cell engraftment. Group 1  
23 received the chemotherapeutic drug cisplatin (3.5 mg/kg) on  
24 treatment days 1, 5 and 9. Group 2 received injections of 25  
25 mg/kg of antibody ARH460-23 three times a week for period of  
26 three weeks. Group 3 received a combination of cisplatin  
27 (3.5 mg/kg) on day 1, 5 and 11 as well as ARH460-23 (25  
28 mg/kg) three times a week for a total of 3 weeks. The control  
29 group (Group 4) received 500  $\mu$ L of 0.9% normal saline three  
30 times a week for a total of three weeks.

31

17.

**1 Study Animal Observation and Endpoints**

2

3 Animals were handled under in accordance with prescribed  
4 practices as outlined by the Canadian Council on Animal Care  
5 (CCAC). All mice were weighed and examined three times a  
6 week for clinical signs of toxicity such as ruffled fur,  
7 lethargy or skin lesions. Once the tumors grew to a size  
8 that was palpable, measurements of tumor length (a) and width  
9 (b) were made with calipers and recorded. Tumor volume was  
10 calculated using the formula:  $V \text{ (tumor volume)} = ab^2/2$ . Tumor  
11 bearing animals were euthanized by CO<sub>2</sub> overdose when the  
12 tumor mass compromised normal behavior/physiology, suffered  
13 severe weight loss or tumors ulcerated according to the CCAC  
14 and University Health Network guidance document on  
15 experimental endpoints.

16

**17 Orthotopic Tumour Engraftment**

18 Green fluorescent protein (GFP)-labelled NCI H460 cells were  
19 injected subcutaneously into five to six week old female NCr-  
20 nu mice to generate stock tumor tissue. When the tumors grew  
21 to log phase, they were harvested and cut into small  
22 fragments of 1 mm<sup>3</sup> each. One fragment of tumor tissue was  
23 then surgically implanted orthotopically into 5-6 week old  
24 female NCr-nu mice.

25

26

27

**28 Treatment**

29 The study consisted of 4 groups of 10 mice. Group 1  
30 received the chemotherapeutic drug cisplatin (3.5 mg/kg) on  
31 treatment days 1, 5 and 9. Group 2 received injections of 25  
32 mg/kg of antibody ARH460-23 three times a week for period of  
33 three weeks. Group 3 received a combination of cisplatin  
34 (3.5 mg/kg) on day 1, 5 and 11 as well as ARH460-23 (25  
35 mg/kg) three times a week for a total of 3 weeks. The control

18

1 group (Group 4) received normal saline three times a week for  
2 a total of three weeks.

3

#### 4 **Study Animal Observation and Endpoints**

5

6 Animal studies were conducted in accordance with the  
7 principles and procedures outlined in the National Institutes  
8 of Health Guide for the Care and Use of Laboratory Animals.  
9 At the end of the study (day 42 post implantation), tumor  
10 measurement of GFP imaging was determined by opening the  
11 thoracic wall. The primary tumors were excised and weighed  
12 at the end of the study.

13 In a xenograft tumor model of lung cancer, ARH460-23  
14 has adjuvant potential when used in combination with  
15 cisplatin. Combination therapy of both ARH460-23 and  
16 cisplatin significantly reduced tumor volume in SCID mice  
17 injected subcutaneously with NCI H460 cells. The  
18 combination treatment also caused a trend towards disease-  
19 free survival.

20 In a xenograft tumor model of lung cancer metastasis,  
21 ARH460-23 significantly inhibited metastasis of NCI H460  
22 tumor tissue following orthotopic transplantation.  
23 ARH460-23 significantly reduced contralateral lung and  
24 thoracic lymph nodes metastasis of GFP-labelled NCI H460  
25 tissue as well as primary tumor size. This work was  
26 performed in collaboration with Anti-Cancer Inc., San  
27 Diego, CA.

#### 28 **ARH460-23 Target Antigen**

29 Keratin 18 (K18), a type I keratin, is a member of  
30 the intermediate filament protein family that exists as an  
31 obligate heterodimer with Keratin 8 (K8), a type II  
32 keratin. The gene was cloned in 1986 and its expression in  
33 normal and cancer tissues has been studied extensively.  
34 K18 and K8 form alpha helical coil-coil filaments that are  
35 10 nm long and attach to the cytosolic nuclear and

1 cellular membrane. Along with other proteins such as actin  
2 and microtubules, the keratins form the cytoskeleton of  
3 many epithelial cells, and staining with antibodies show  
4 that K18 is distributed in mammary cells, hepatocytes, and  
5 epidermal cells, among others. There is increased  
6 expression of K18 in many carcinomas including breast  
7 cancer, transitional cell carcinoma, hepatocellular  
8 carcinoma, pancreatic adenocarcinoma, colon adenocarcinoma  
9 and prostate cancer. However, nonepithelial cancer can  
10 also have aberrant K18 expression such as in the case of  
11 melanoma, and lymphoma.

12       The normal function of intracellular simple  
13 epithelial keratins may be to provide mechanical strength  
14 to the cells, but there may also be less well-defined  
15 functions. K18 may be involved in signal transduction  
16 through interactions with protein kinase C, or through  
17 interactions with the desmosome. There is some evidence  
18 that K18 is involved in apoptosis as a target of caspases  
19 when activated by other apoptotic stimuli and in  
20 supporting resistance to FAS-mediated apoptosis in  
21 hepatocytes.

22       The following table underscores the distribution of  
23 K18 in various cancers.

Table 1: K18 Distribution in Cancer

Cancer Type	N	Incidence	Source
Warthins tumor	26	100.0	Schwerer, Histopathology: 347, 2001
Wilms tumor	9	88.9	Rebhandl, Med Pediatr Oncol: 357, 2001
Squamous cell carcinomas of the oesophagus	35	97.0	Lam, Virchows Arch: 345, 1995
Poorly differentiated thyroid carcinoma	153	60.0	Lam, Eur J Surg Oncol: 631, 2001
Anaplastic thyroid carcinoma	153	80.0	Lam, Eur J Surg Oncol: 631, 2001
Medullary thyroid carcinoma	153	85.0	Lam, Eur J Surg Oncol: 631, 2001
Breast: medullary carcinoma	31	100.0	Lam, Eur J Surg Oncol: 631, 2001
Cholangiocarcinoma	77	77.0	Shimonishi, Histopathology: 55, 2000
	min		
Epithelioid hemangioendotheliomas	137	100.0	Miettinen, Hum Pathol: 1062, 2000
	min		
Epithelioid angiosarcomas	137	50.0	Miettinen, Hum Pathol: 1062, 2000
	min		
Angiosarcomas	137	20.0	Miettinen, Hum Pathol: 1062, 2000
Synovial sarcoma	110	100.0	Miettinen, Virchows Arch: 275, 2000
Pancreatic carcinoma	48	8.3	Thorban, Ann Oncol: 111, 1999
Squamous cell carcinoma	26		Depondt, Eur J Oral Sci: 442, 1999
Hepatocellular carcinoma	30	100.0	Tsuji, Pathol Int: 310, 1999
Cholangiocarcinoma	10	100.0	Tsuji, Pathol Int: 310, 1999
Infiltrating ductal breast carcinoma	101	majority	Malzahn, Virchows Arch: 119, 1998
Invasive ductal breast carcinoma	100	100.0	Rejthar, Neoplasma: 370, 1997
Hepatocellular carcinoma	20	55.0	D'Errico, Hum Pathol: 599, 1996
Cholangiocarcinoma	15	80.0	D'Errico, Hum Pathol: 599, 1996
Prostate carcinoma	84	33.0	Oberneder, Urol Res: 3, 1994
Prostate carcinoma	13	38.5	Riesenberg, Histochemistry: 61, 1993
Breast or gastrointestinal adenocarcinoma	532	33.0	Pantel, J Natl Cancer Inst: 1419, 1993
Melanoma	52	48.1	Fuchs, Am J Pathol: 169, 1992
Renal cell carcinoma	30	100.0	Dierick, Histopathology: 315, 1991
Hepatocellular carcinoma	34	100.0	Van Eyken, Hum Pathol: 562, 1988

1

2           There is also evidence for the extracellular  
3 expression of keratins. For example K18 has been  
4 identified as a hepatic receptor for thrombin-anti-  
5 thrombin complexes, and K8 on hepatocytes has been shown  
6 to bind plasminogen and tissue plasminogen activator.  
7 There is speculation that the extracellular expression of  
8 keratins may contribute to increased invasiveness in  
9 cancer.

21

1        Although K18 is well defined as a cancer marker, its  
2        role in the oncogenesis is less certain. Transfection of  
3        K8/K18 into melanoma increased their metastatic potential  
4        and their invasiveness, but this was not universal for all  
5        cancer types. Epithelium K8/K18 may provide resistance to  
6        Fas-mediated apoptosis and studies have found that the  
7        expression of K8 and K18 confer multiple drug resistance.

8        ARH460-23 is a monoclonal antibody that targets K18  
9        and is being developed for therapeutic use in cancer. The  
10       commercial attractiveness of the antibody is underscored  
11       by the high percentage of cancers that express the H460-23  
12       antigen. The predominant expression of the antigen on the  
13       cell surface of cancers and not on normal cells suggests  
14       the biology of the antigen is specifically drugable.

15

#### 16    **Biochemical Identification of the H460-23 Antigen**

17       Identification of the H460-23 antigen involved two-  
18       dimensional electrophoresis and Western blotting (See  
19       Figs. 3 and 4). Membranes were prepared from ARH460-23  
20       high (NCI H460) and ARH460-23 low (Jurkat) cell lines and  
21       analyzed with 2-D polyacrylamide gel electrophoresis to  
22       separate proteins according to their molecular weight and  
23       pH. The goal was to isolate a spot from the NCI H460  
24       membrane proteins that was uniquely reactive with ARH460-  
25       23. Antibody 11E10 was used as a control.

26       ARH460-23 uniquely identified one protein spot from  
27       the NCI H460 membrane proteins. The corresponding protein  
28       to the one recognized on the Western blot was identified  
29       on the Sypro-stained gel. The spot had a molecular weight  
30       of 47.3 kDa and pI of 5.3. The protein was robotically  
31       spotted, excised from the gel, and digested with trypsin  
32       for matrix-assisted laser desorption/ionization (MALDI)/  
33       mass spectrometry (MS) analysis. The MALDI/MS data were  
34       submitted to Profound (Proteometrics software package) for  
35       peptide mass fingerprint searching. Twenty-six of the 45

22

1 peptides generated from the spot matched cytokeratin 18.  
2 The peptides generated had 65% minimum sequence coverage  
3 of cytokeratin 18. These results indicate that  
4 cytokeratin 18 is likely the putative H460-23 antigen.  
5

#### 6 **ARH60-23 Binding to Human Cancer Cell Lines**

7 ARH460-23 binding was evaluated in 21 human cancer  
8 cell lines using flow cytometry to explore the  
9 distribution of the ARH460-23 target antigen across a  
10 range of human cancers. Antibodies of the same isotype,  
11 11E10 was used as a negative control and antibody EOS9.1  
12 which recognizes the CD95 antigen was used to probe  
13 whether the ARH460 target is CD95.  
14 Figure 5 shows histogram profiles, and Table 2 summarizes  
15 H460-23 staining relative to the controls. The lung cell  
16 line, NCI H460, and umbilical vein epithelial cells, HUV-  
17 EC-C, show a high level of ARH460-23 expression compared  
18 to the other cell lines in this group. Overall, the  
19 majority of the solid tumors tested showed moderate levels  
20 of H460-23 antigen expression while the hematologic  
21 cancers, leukemia Jurkat and K562 and the T cell lymphoma  
22 K3P exhibited only marginal levels. The large cell lung  
23 carcinoma cell line NCI H661, unlike the NCI H460 cells,  
24 exhibited weak H460-23 staining. These findings suggest  
25 that ARH460-23 recognizes an antigen that is distributed  
26 differently on different types of cancer.

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TABLE 2 Flow Cytometric Analysis of Cell Lines Stained with ARH460-23

Cell Line	Description	H460-23 Staining	EOS9.1 Staining
NCI H460	Large Cell Lung Cancer	Very Strong	Positive
Jurkat	Acute T cell Leukemia	Weak	Positive
K562	Chronic Myelogenous Leukemia	Weak	Negative
K3P	T cell Lymphoma	Weak	Positive
NCI H661	Large Cell Lung Cancer	Weak	Negative
HEY	Ovarian Epithelial Cancer	Strong	Positive
AU565	Mammary adenocarcinoma	Medium	Positive
T-47D	Mammary Ductal Carcinoma	Strong	Positive
MCF-7	Mammary Adenocarcinoma	Strong	Negative
EBV-transfected lymphocytes patient # 14725	Epstein Barr virus-in vitro transfected cells	Weak-Medium	Positive
EBV-transfected lymphocytes patient # 14935	Epstein Barr virus-in vitro transfected cells	Weak	Positive
HUV-EC-C	Human umbilical vein endothelial cells	Very Strong	Positive
A2058	Melanoma	Weak-Medium	Positive
Hs574.t	Mammary ductal carcinoma	Weak -Medium	Positive
Hs.888 Lu	Normal lung	Medium	N.D.
Hs574.Mg	Normal mammary	Medium	N.D.
SK-BR3	Mammary adenocarcinoma	Medium	Positive
CCD.27sK	Normal skin	Medium	N.D.
NCI-N87	Gastric carcinoma	Medium	Positive
BT-549	Mammary epithelial cancer	Medium	Positive
BxPC-8	Pancreatic adenocarcinoma	Strong	Positive

**1 Target Validation**

2 An immunohistochemistry study was carried out on  
3 formalin-fixed, paraffin-embedded human tissues by Qualtek  
4 Molecular Laboratories of Santa Barabara, CA to profile  
5 expression of the ARH460-23 antigen on normal and tumor  
6 tissues, and is summarized in Figure 7. Normal mouse  
7 serum was run in parallel as a negative control. Scoring  
8 of the specific antibody reactivity was done on the  
9 standard pathology scale of 0 to 4. Whenever possible the  
10 tissue was scored on a subcellular level to indicate  
11 reactivity within the nucleus, cytoplasm and plasma  
12 membrane.

13 Most tumor tissues tested were strongly positive for  
14 ARH460-23. In the colon and prostate all tumors were  
15 highly reactive and found to have both membranous and  
16 cytoplasmic binding. The one ovarian carcinoma tested  
17 yielded a similar result.

18 Lung and breast carcinomas and melanomas yielded more  
19 mixed results. In the lung, both large cell carcinomas  
20 were highly reactive on the cell membrane and in the  
21 cytoplasm. Of the 2 adenocarcinomas examined, one was  
22 positive for ARH460-23 and one negative. In breast, 7 of 9  
23 carcinomas were positive. In medulary carcinomas where  
24 lymphocytes were present, the lymphocytes were also  
25 positive for ARH460-23. Two of the 4 melanomas tested were  
26 positive and reactive in both the cell membrane and  
27 cytoplasm.

28 In normal breast, skin, lung and ovary epithelia,  
29 ARH460-23 was either positive or weakly positive but  
30 confined to the cytoplasm and perinuclear regions of the  
31 cell. In both colon and ovary the normal tissues tested  
32 were negative for ARH460-23. However, normal colon  
33 displayed inflammatory cell reactivity in the stroma that  
34 was both membranous and cytoplasmic. When normal tissues  
35 were positive it was mainly confined to the cytoplasm and

25

1 perinuclear membranes. Typically when a tumor was positive  
2 for ARH460-23 it was found to be both reactive in the  
3 membrane and cytoplasm (16 of 18 or 89%). In addition,  
4 inflammatory cells found in proximity to tumor were  
5 reactive to ARH460-23 on the cell membrane and in the  
6 cytoplasm.

7 It was concluded that the antibody shows reactivity  
8 in both normal and neoplastic cells as well as populations  
9 of inflammatory cells in the colon and in proximity to  
10 tumors. The antibody tends to be reactive to cell  
11 membranes in many tumors and in lymphocytes but in normal  
12 epithelia it is mainly confined to cytoplasm and  
13 perinuclear regions.

14 All patents and publications mentioned in this  
15 specification are indicative of the levels of those  
16 skilled in the art to which the invention pertains. All  
17 patents and publications are herein incorporated by  
18 reference to the same extent as if each individual  
19 publication was specifically and individually indicated to  
20 be incorporated by reference.

21 It is to be understood that while a certain form of  
22 the invention is illustrated, it is not to be limited to  
23 the specific form or arrangement of parts herein described  
24 and shown. It will be apparent to those skilled in the  
25 art that various changes may be made without departing  
26 from the scope of the invention and the invention is not  
27 to be considered limited to what is shown and described in  
28 the specification. One skilled in the art will readily  
29 appreciate that the present invention is well adapted to  
30 carry out the objects and obtain the ends and advantages  
31 mentioned, as well as those inherent therein. Any  
32 oligonucleotides, peptides, polypeptides, biologically  
33 related compounds, methods, procedures and techniques  
34 described herein are presently representative of the  
35 preferred embodiments, are intended to be exemplary and

26

1 are not intended as limitations on the scope. Changes  
2 therein and other uses will occur to those skilled in the  
3 art which are encompassed within the spirit of the  
4 invention and are defined by the scope of the appended  
5 claims. Although the invention has been described in  
6 connection with specific preferred embodiments, it should  
7 be understood that the invention as claimed should not be  
8 unduly limited to such specific embodiments. Indeed,  
9 various modifications of the described modes for carrying  
10 out the invention which are obvious to those skilled in  
11 the art are intended to be within the scope of the  
12 following claims.

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CLAIMS

What is claimed is:

Claim 1. A method of treating a human tumor in a mammal, wherein said tumor expresses an antigen which specifically binds to a monoclonal antibody or antigen binding fragment thereof which has the identifying characteristics of a monoclonal antibody encoded by a clone deposited with the ATCC as accession number PTA-2700 comprising administering to said mammal said monoclonal antibody in an amount effective to reduce said mammal's tumor burden.

Claim 2. The method of claim 1 wherein said antibody is conjugated to a cytotoxic moiety.

Claim 3. The method of claim 2 wherein said cytotoxic moiety is a radioactive isotope.

Claim 4. The method of claim 1 wherein said antibody activates complement.

Claim 5. The method of claim 1 wherein said antibody mediates antibody dependent cellular cytotoxicity.

Claim 6. The method of claim 1 wherein said antibody is a murine antibody.

Claim 7. The method of claim 1 wherein said antibody is a humanized antibody

Claim 8. The method of claim 1 wherein said antibody is a chimerized antibody.

Claim 9. An isolated monoclonal antibody or antigen binding fragments thereof encoded by the clone deposited with the ATCC as PTA-2700.

Claim 10. The isolated antibody or antigen binding fragments of claim 9, wherein said isolated antibody or antigen binding fragments thereof is humanized.

Claim 11. The isolated antibody or antigen binding fragments of claim 9 conjugated with a member selected from the group consisting of cytotoxic moieties, enzymes, radioactive compounds, and hematogenous cells.

Claim 12. The isolated antibody or antigen binding fragments of claim 9, wherein said isolated antibody or antigen binding fragments thereof is a chimerized antibody.

Claim 13. The isolated antibody or antigen binding fragments of claim 9, wherein said isolated antibody or antigen binding fragments thereof is a murine antibody.

Claim 14. The isolated clone deposited with the ATCC as PTA-2700.

Claim 15. A binding assay to determine presence of cancerous cells in a tissue sample selected from a human tumor comprising:

providing a tissue sample from said human tumor ;

providing an isolated monoclonal antibody or antigen binding fragment thereof encoded by the clone deposited with the ATCC as PTA-2700;

contacting said isolated monoclonal antibody or antigen binding fragment thereof with said tissue sample; and

determining binding of said isolated monoclonal antibody or antigen binding fragment thereof with said tissue sample;

whereby the presence of said cancerous cells in said tissue sample is indicated.

Claim 16. The binding assay of claim 15 wherein the human tumor tissue sample is obtained from a tumor originating in a tissue selected from the group consisting of colon, ovarian, lung, and breast tissue.

Claim 17. A process of isolating or screening for cancerous cells in a tissue sample selected from a human tumor comprising:

providing a tissue sample from a said human tumor ;

providing an isolated monoclonal antibody or antigen binding fragment thereof encoded by the clone deposited with the ATCC as PTA-2700;

contacting said isolated monoclonal antibody or antigen binding fragment thereof with said tissue sample; and

determining binding of said isolated monoclonal antibody or antigen binding fragment thereof with said tissue sample;

whereby said cancerous cells are isolated by said binding and their presence in said tissue sample is confirmed.

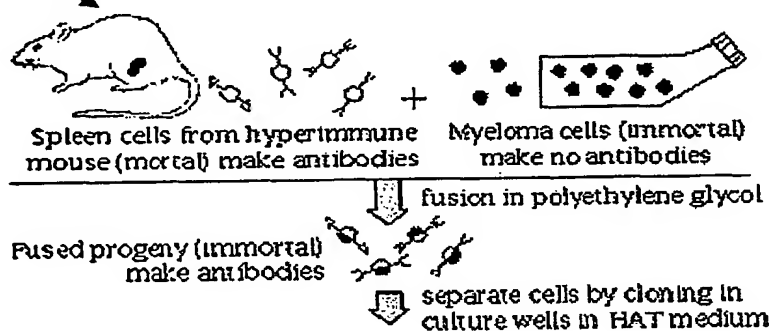
Claim 18. The process of claim 17 wherein the human tumor tissue sample is obtained from a tumor originating in a tissue selected from the group consisting of colon, ovarian, lung, and breast tissue.



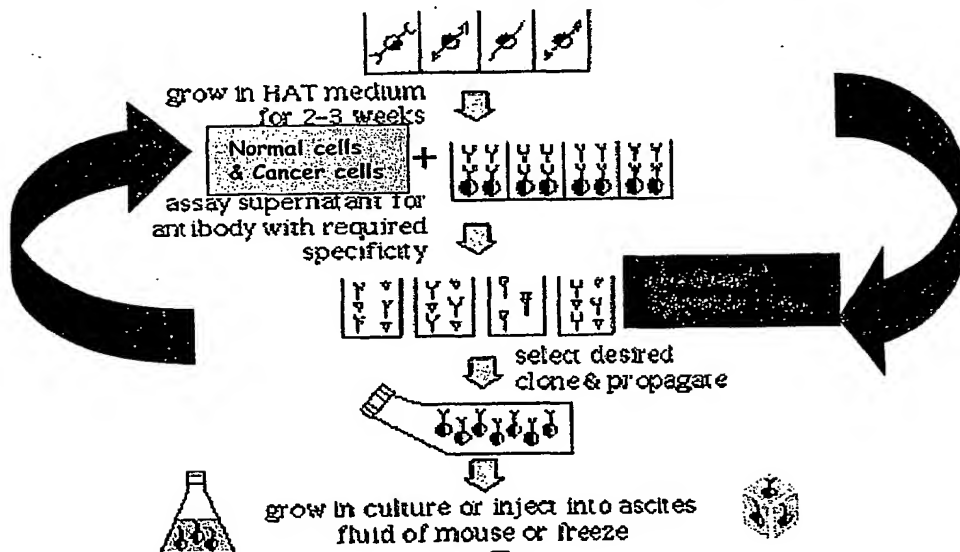
# Strategy For Anti-Cancer Antibody Generation

## Tumor Injection

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## Hybridoma Generation



## Functional Screening Strategy

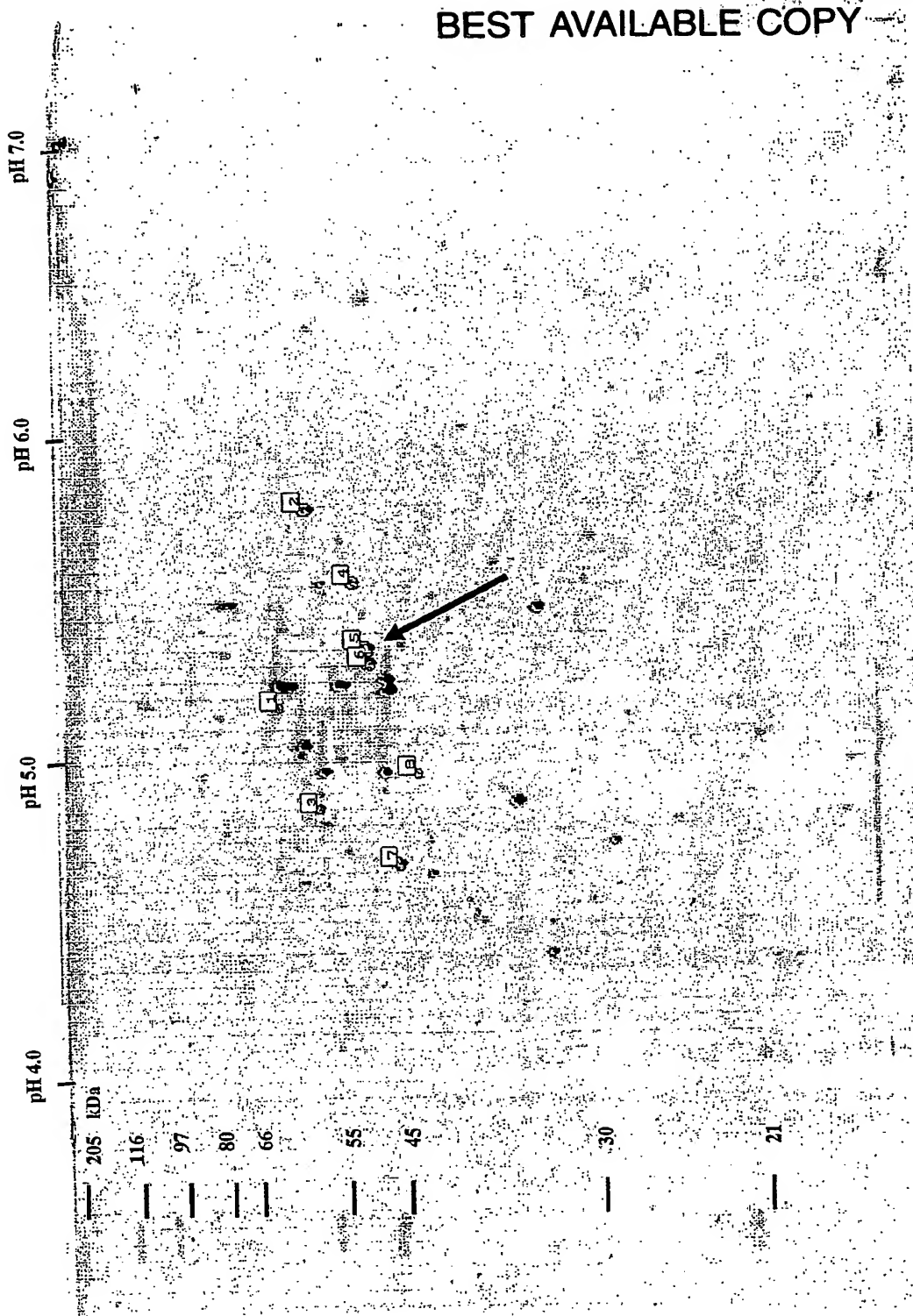
## Antibody Purification

**Identify Target Antigen****In Vivo Tumor Studies**

## ARH460-23 Alone Significantly Reduced Metastasis of Lung Tumor (NCI H460) In Orthotopic Implantation Model

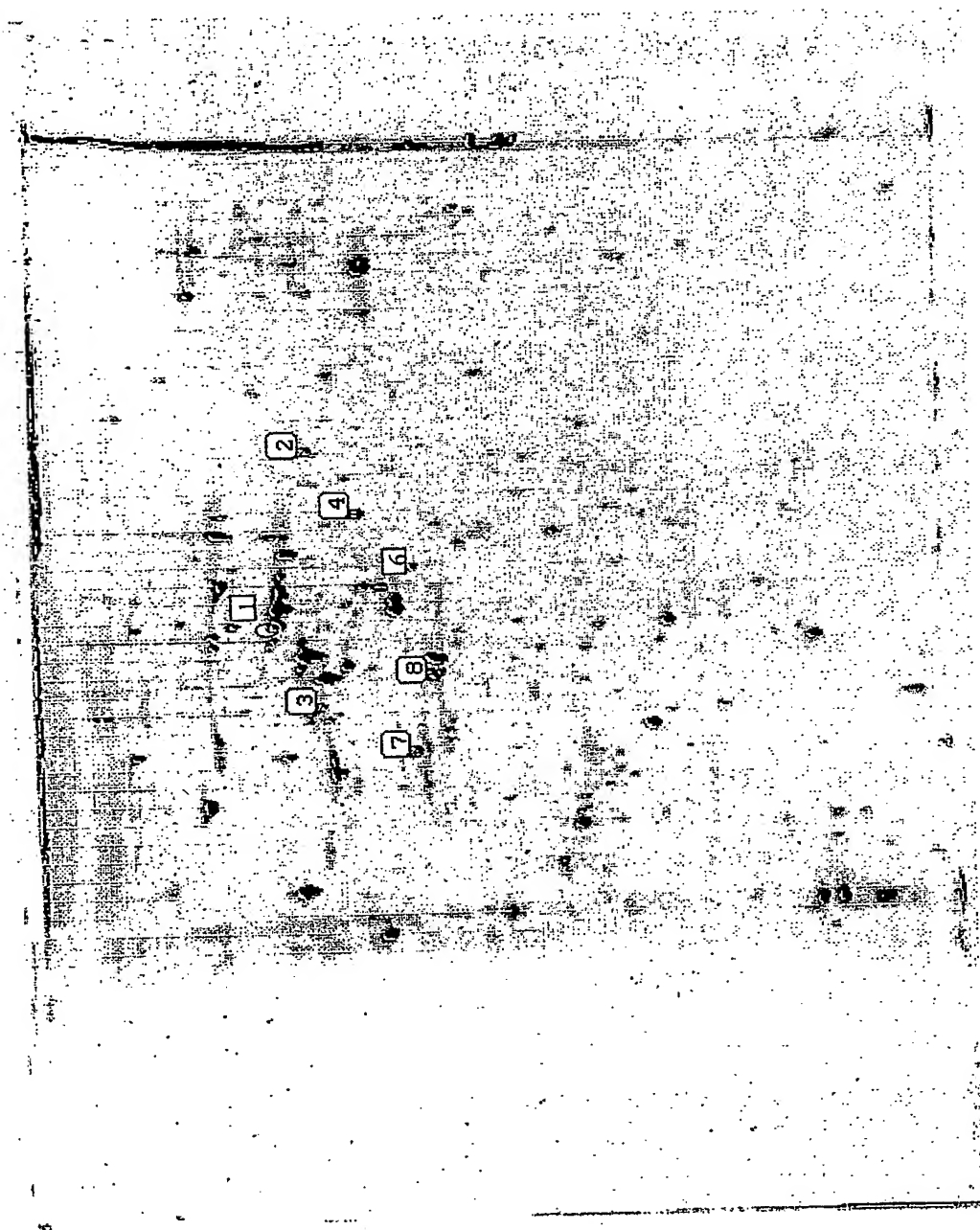
Group	Mouse	Metastasis and sites		Final primary tumor weight (g)	
		Contralateral lung	Thoracic LN	Mean	SD
Cisplatin 3.5 mg/kg	Positive	6/9	7/9	1.52	0.36
	P value vs. control	1	1	0.27	
ARH460-23A 25 mg/kg	Positive	3/10	2/10	1.23	0.25
	P value vs. control	0.074	0.007	0.023	
Cisplatin 3.5 mg/kg and ARH460-23A 25 mg/kg	Positive	5/9	4/9	1.38	0.55
	P value vs. control	0.650	0.170	0.084	
Control	Positive	7/10	8/10	1.99	0.72

NCr-nu mice were treated with antibody and/or cisplatin following orthotopic implantation of GFP-labelled NCI H460 cells. Mice were given ARH460-23 at 3 days post implantation and cisplatin at 1, 5, and 9 days post implantation. Primary tumor weights were recorded and tumor metastasis to the contralateral lung and thoracic lymph nodes (LN) was determined at 42 days post implantation. This work was done in collaboration with Anti-Cancer Inc., San Diego, CA.



2-D gel analysis of NCI H460 membrane proteins probed with ARH460-23. Membrane lysates were run on 2-D gels and stained with sypro ruby (gel above) or transferred to a blot for probing with ARH460-23. Spot 5 (see arrow) was uniquely reactive to ARH460-23 as this spot was not detectable in a 2-D blot of Jurkat membrane proteins. By matrix-assisted laser desorption/ionization/mass spectrometry analysis, this spot was identified as human cytokeratin 18.

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2-D gel analysis of membrane proteins from Jurkat cells probed with ARH460-23. Jurkat cell membrane lysates were run on 2-D gels and stained with sypro ruby (gel above) or transferred to a membrane blot and probed with ARH460-23. Spot 5 (cytokeratin 18) was not detected in the 2-D blot.

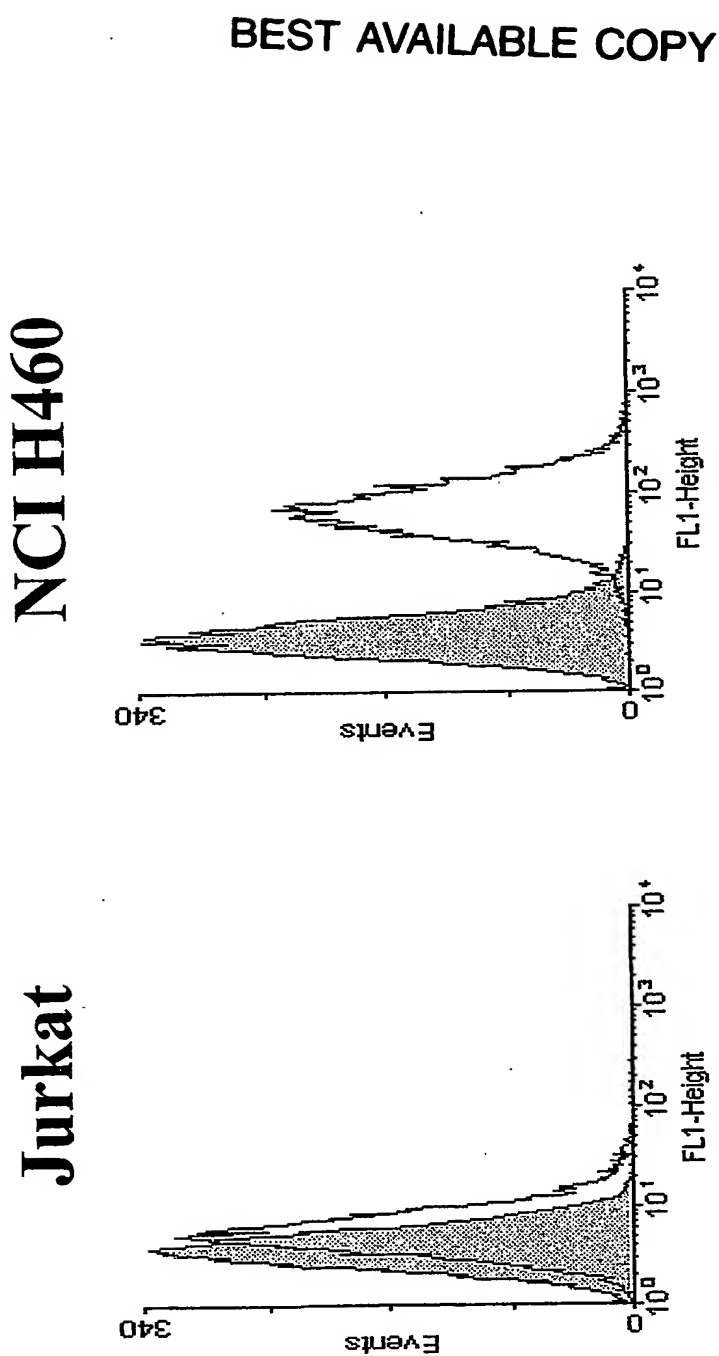
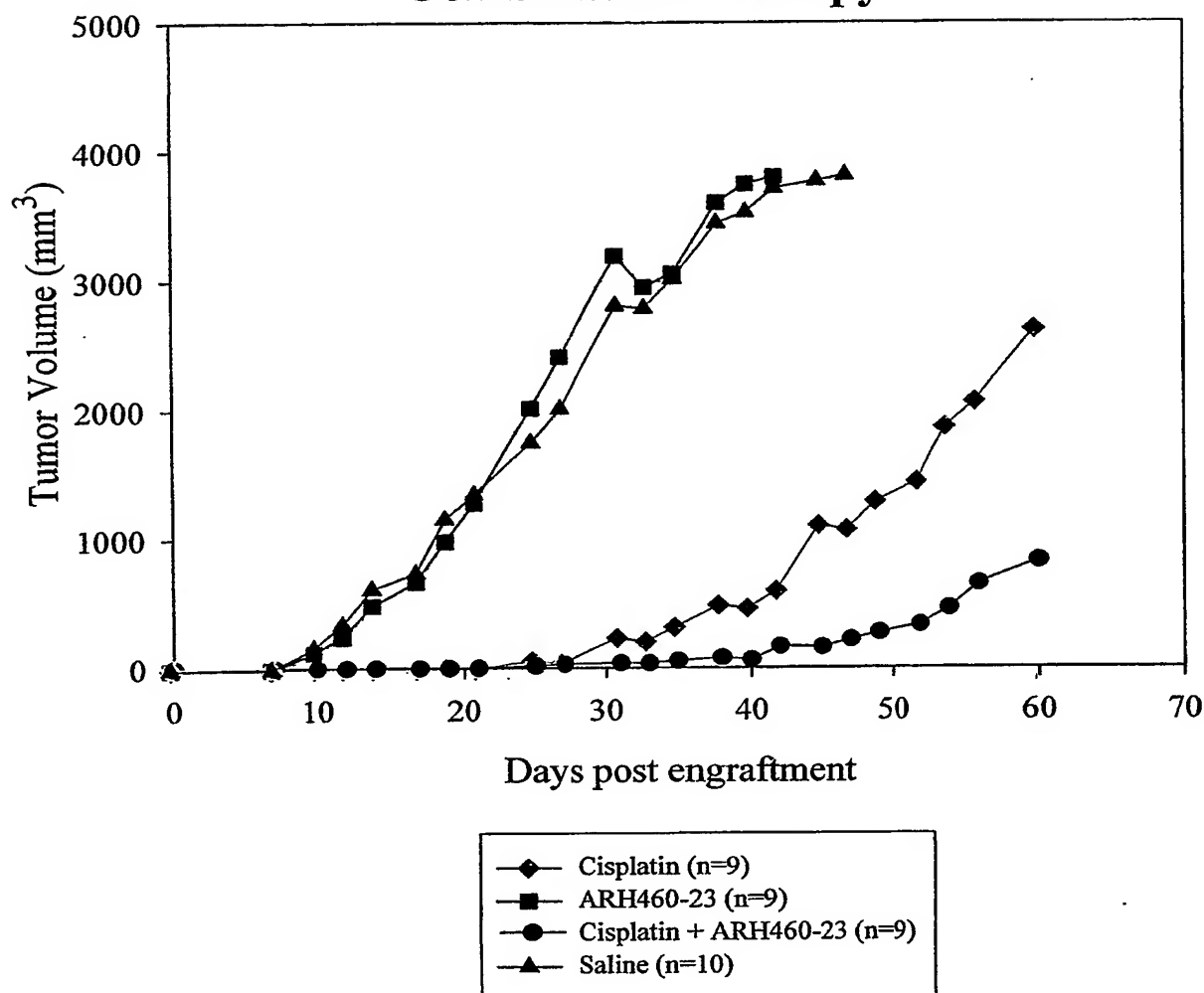


Figure. Differential reactivity of ARH460-23 for Jurkat and NCI H460 cells. Cells were stained with either 2  $\mu$ g of purified ARH460-23 or the isotype control mouse IgM antibody 11E10 followed by Alexa Fluor 488 conjugated goat anti-mouse IgM. The histogram plots of isotype control staining (shaded) are compared with ARH460-23 staining (dark outline).

### Tumour Growth Kinetics Cisplatin/ARH460-23 Preventative Combination Therapy



The effect of preventative therapy on tumour growth kinetics. Average tumor volumes are plotted as a function of time. Combination treatment of cisplatin and ARH460-23 significantly reduced the tumor growth rate ( $p < 0.05$ ).

7/8

## Immunohistochemistry staining results with Arius H460-23 (QML Project # 419)

Results from 20 minute SHIER / no enzyme tissue pretreatment (overnight H460-23 incubation at 3.0µg/ml)

TISSUE TYPE	SAMPLE NO.	TISSUE PATHOLOGY	Reactivity	Cellular Stain location	Stain Scale 1-4	Mouse IgG Control	Comments
COLON	1	NORMAL epithelial glands	NEGATIVE			NEGATIVE	
	2	CARCINOMA	POSITIVE	Membranous, perinuclear and cytoplasm	4	NEGATIVE	
		NORMAL ADJACENT	NEGATIVE				
	3	CARCINOMA	POSITIVE	Membranous, perinuclear and cytoplasm	4	NEGATIVE	Inflammatory cells also positive
		NORMAL ADJACENT	NEGATIVE				
PROSTATE	4	NORMAL	POSITIVE	Cytoplasm	4	NEGATIVE	
	5	CARCINOMA	POSITIVE	Membranous, perinuclear and cytoplasm	4	NEGATIVE	Inflammatory cells also positive
	6	CARCINOMA	POSITIVE	Membranous, perinuclear and cytoplasm	4	NEGATIVE	Inflammatory cells also positive
	7	CARCINOMA GLEASON GRADE 5	POSITIVE	Membranous, perinuclear and cytoplasm	4	NEGATIVE	Inflammatory cells also positive
LUNG	8	NORMAL	POSITIVE	Cytoplasm and perinuclear	4	NEGATIVE	Bronchial Columnar Epithelial cells negative
	9	ADENOCARCINOMA	NEGATIVE		2	NEGATIVE	Inflammatory cells also positive
	10	ADENOCARCINOMA	POSITIVE	Membranous and cytoplasm	4	NEGATIVE	Inflammatory cells also positive
	11	LARGE CELL CARCINOMA	POSITIVE	Membranous and cytoplasm	4	NEGATIVE	Inflammatory cells also positive
	12	LARGE CELL CARCINOMA	POSITIVE	Membranous and cytoplasm	4	NEGATIVE	Inflammatory cells also positive
OVARY	13	NORMAL	NEGATIVE			NEGATIVE	Stroma light
	14	CARCINOMA	POSITIVE	Membranous and cytoplasm	4	NEGATIVE	Stroma Positive
SKIN	15	NORMAL	POSITIVE	Cytoplasm and perinuclear	4	NEGATIVE	
	16	MELANOMA - Metastasis to breast	POSITIVE	Cytoplasm	4	NEGATIVE	Inflammatory cells also positive
	17	NODULAR MELANOMA	POSITIVE	Membranous and cytoplasm	4	NEGATIVE	Positive melanin Melanocytes
	18	MELANOMA - residual recurrent invasive melanoma	NEGATIVE			NEGATIVE	
	19	MELANOMA - trabecular with lymphatic involvement	NEGATIVE			NEGATIVE	
BREAST	20	NORMAL	WEAKLY POSITIVE	Cytoplasm	2	NEGATIVE	
	21	DUCTAL INVASIVE CARCINOMA Moderate to poorly differentiated	WEAKLY POSITIVE	Membranous and cytoplasm	3	NEGATIVE	
	22	DUCTAL INVASIVE CARCINOMA well to moderately differentiated	NEGATIVE			NEGATIVE	
	23	DUCTAL INVASIVE CARCINOMA moderately differentiated	NEGATIVE			NEGATIVE	
	24	DUCTAL INVASIVE CARCINOMA poorly differentiated	POSITIVE	Cytoplasm and perinuclear	4	NEGATIVE	
	25	MEDULARY CARCINOMA well differentiated	POSITIVE	Membranous and cytoplasm	3	NEGATIVE	Inflammatory cells also positive
	26	DUCTAL INVASIVE CARCINOMA well to moderately differentiated	NEGATIVE			NEGATIVE	
	27	DUCTAL INVASIVE CARCINOMA well to moderately differentiated	POSITIVE	Membranous and cytoplasm	4	NEGATIVE	
	28	NORMAL	WEAKLY POSITIVE	Cytoplasm	2	NEGATIVE	
	29	DUCTAL INVASIVE CARCINOMA well to moderately differentiated	POSITIVE	Membranous and cytoplasm	4	NEGATIVE	
	30	HYPERPLASIA	POSITIVE	Cytoplasm	4	NEGATIVE	
	31	MEDULARY CARCINOMA well differentiated	POSITIVE	Membranous and cytoplasm	4	NEGATIVE	Inflammatory cells also positive
CONTROL CELL PELLETS	32	NCI-H360 Cells	POSITIVE	Membranous and cytoplasm	4	NEGATIVE	Figure 1
	33	Jurkat Cells	NEGATIVE			NEGATIVE	Figure 1

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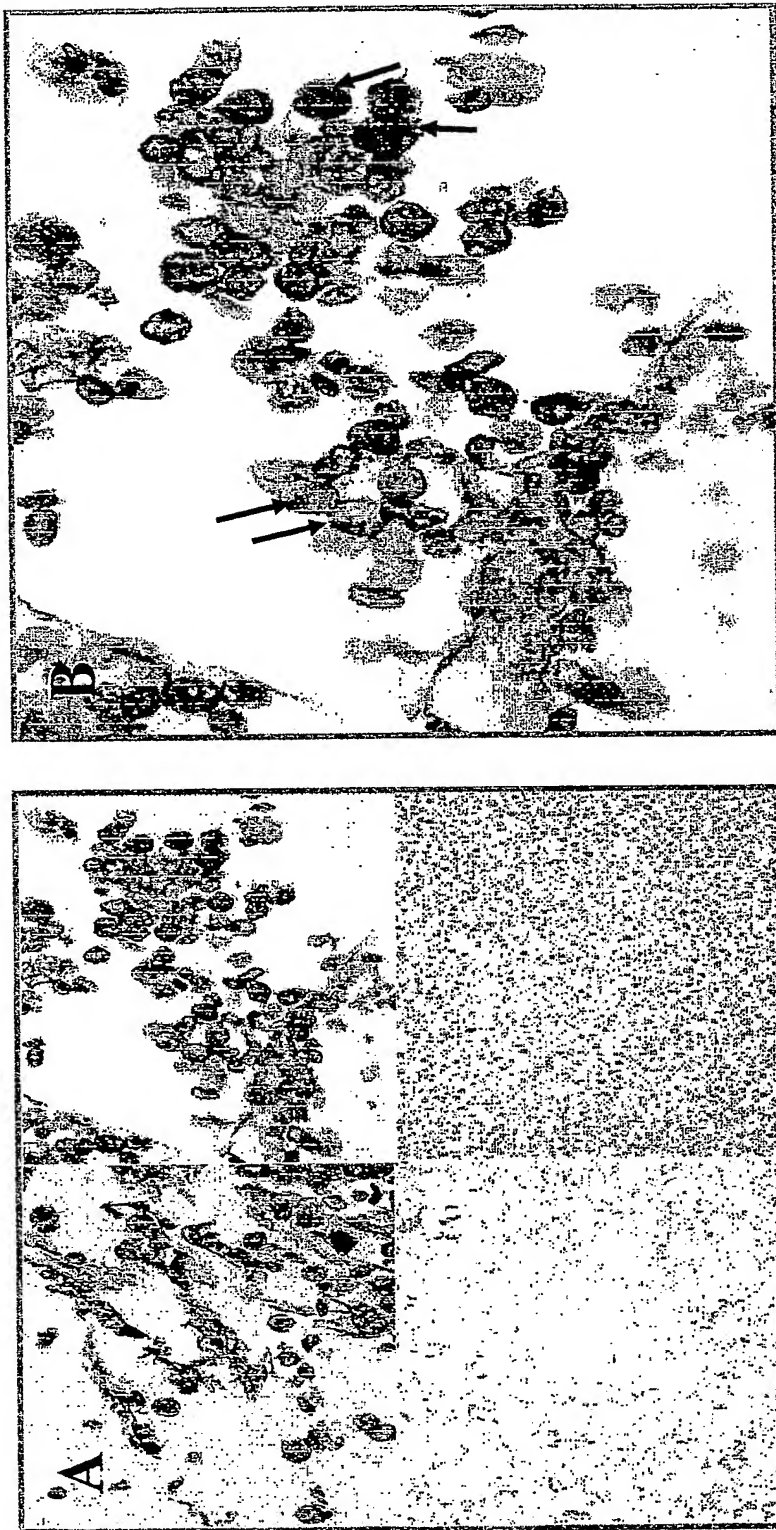


Figure A. Immunohistochemical staining of paraffin-embedded, formalin-fixed NCI H460 (top) and Jurkat (bottom) cell pellets with ARH460-23. ARH460-23 specifically reacted with NCI H460 cells.

Figure B. Immunohistochemical staining of paraffin-embedded, formalin-fixed NCI H460 (top) cell pellets with ARH460-23. ARH460-23 reacted with both the cell membrane (black arrows) and cytoplasm (blue arrows) of NCI H460 cells.



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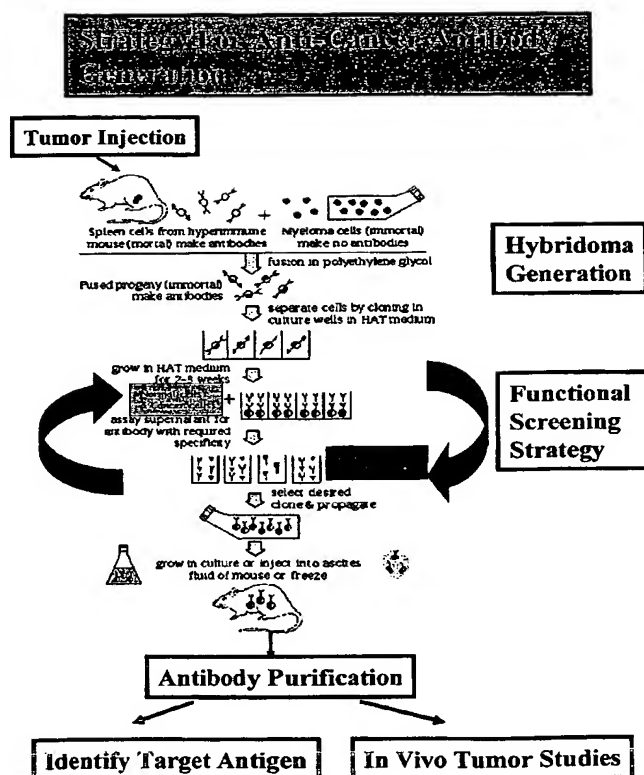
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[Continued on next page]

(54) Title: **ANTI-CK18 MONOCLONAL ANTIBODY AND THERAPEUTIC AND DIAGNOSTIC USES THEREOF IN CANCER**



(57) Abstract: The present invention relates to a method for producing patient specific anti-cancer antibodies using a novel paradigm of screening and characterizing the target antigen thereof. The invention further relates to the process by which the antibodies are made and to their methods of use. The antibodies can be used in aid of staging and diagnosis of a cancer, and can be used to treat primary tumors and their metastases. The anti-cancer antibodies can be combined with a variety of anti-neoplastic agents, e.g. Cisplatin, to derive a synergistic effect with regard to reduction in tumour growth kinetics and metastasis.

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## A. CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

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Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CANCERLIT, MEDLINE, BIOSIS, EMBASE, EPO-Internal, WPI Data, PAJ

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ETO HIROSHI ET AL: "Mapping and regulation of the tumor-associated epitope recognized by monoclonal antibody RS-11." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, no. 35, 1 September 2000 (2000-09-01), pages 27075-27083, XP002254882 ISSN: 0021-9258 page 27075, right-hand column, line 32-48 page 27082, left-hand column, line 55 -right-hand column, line 14 ---	1-18
X	WO 95 31728 A (BEKI AB ;BJOERKLUND PETER (SE); RYDLANDER LARS (SE)) 23 November 1995 (1995-11-23) page 7, line 21 -page 8, line 5 page 9, line 22-26 examples 3-6 --- -/--	9-18

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

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Date of mailing of the international search report

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SASAKI M ET AL: "Immunoperoxidase staining for cytokeratins 8 and 18 is very sensitive for detection of occult node metastasis of colorectal cancer: A comparison with genetic analysis of K-ras." HISTOPATHOLOGY (OXFORD), vol. 32, no. 3, March 1998 (1998-03), pages 199-208, XP002254883 ISSN: 0309-0167 abstract page 201, left-hand column, paragraph 2 ----	9-18
A	US 6 180 357 B1 (YOUNG DAVID S F ET AL) 30 January 2001 (2001-01-30) the whole document ----	1-18
E	WO 03 057168 A (JENSENIUS JENS C ;SCRIPPS RESEARCH INST (US); DITZEL HENRIK (US)) 17 July 2003 (2003-07-17) page 4, line 13 -page 5, line 6 page 66, line 13-15 -----	1-18

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/CA 03/00500

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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Although claims 1-8 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
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## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

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- ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

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